STRUCTURE AND COMPOSITION OF CITRUS LEAVES AFFECTED WITH MESOPHYLL COLLAPSE[1]

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(WITH SIX FIGURES)

Introduction

A pathological condition not infrequently observed in leaves of orange trees as yellow, translucent, sunken areas, has been called "mesophyll collapse" by FAWCETT (**6**). Neither bacteria nor fungi seem to initiate this disorder of the leaves, though they may invade the weakened and collapsed tissues secondarily. The disorder has therefore been regarded as a physiological disturbance caused by insects and by edaphic and climatic factors, or by edaphic or climatic factors alone.

FAWCETT (**6**) first found mesophyll collapse in the coastal districts of southern California. In a series of papers, FAWCETT *et al.* outlined the distribution of mesophyll collapse in South America. They found it in the coastal areas of the state of Rio de Janeiro, Brazil (**11**), but did not find it two hundred miles in the interior, in the state of Minas Geraes (**12**). It was also found fairly near the coast, in the states of Pernambuco (**7**) and Bahia (**8**), and in Sao Paulo as far as 40 miles inland and at elevations as high as 2,500 feet above sea level (**8**). None was noted in the Pitangueires area, some two hundred miles from the sea coast; in the state of Rio Grande do Sul, however, it was found near the coast, at Montenegro (**8**). It was noted at Uruguayana, somewhat inland, near the Uruguay River (**8**), as well as at Concordia, Argentina, also near the Uruguay River. These investigators, however, found no mesophyll collapse in any other parts of Argentina (**9**). In Paraguay, mesophyll collapse was found at Asuncion, near the Paraguay River (**10**).

The writers have made observations in North America and have noted the occurrence of mesophyll collapse in the coastal districts of the United States and Mexico. We have found it in the coastal districts of Florida, near Vero Beach, and in the coastal valleys of California. It has been observed in Arizona[2] and it probably exists, also, in some parts of Texas near the gulf. Our survey in California has shown that the disorder is present in Ventura, Santa Barbara, Los Angeles, San Bernardino, Riverside, Orange, and San Diego counties. Its general distribution in California is shown in figure 1.

This leaf condition is most readily recognized when the mature leaves are examined by transmitted light. It will then be observed that yellow, translucent areas lacking chlorophyll occur principally in the central portion of the blade, on one or both sides of the midrib (plate I, fig. 2). By reflected

[1] Paper no. 484, University of California Citrus Experiment Station, Riverside, California.

[2] Observation reported by DR. R. B. STREETS, Associate Plant Pathologist, University of Arizona, Tucson, in letter to the senior author, dated March 1, 1943.

light these areas on the lower surface of the leaf appear grayish and somewhat sunken below the normal leaf surface. Necrosis or browning of the translucent areas may also occur. The leaves, however, are otherwise apparently normal, as are the twigs on which they are carried. So far, the symptoms have been observed on Valencia and Washington Navel oranges, but not on lemons.

The disorder is of interest because it represents one of the foliar symptoms for which explanations have long been sought. HAAS (**14**) has suggested that a water deficit during the hot summer months may be involved,

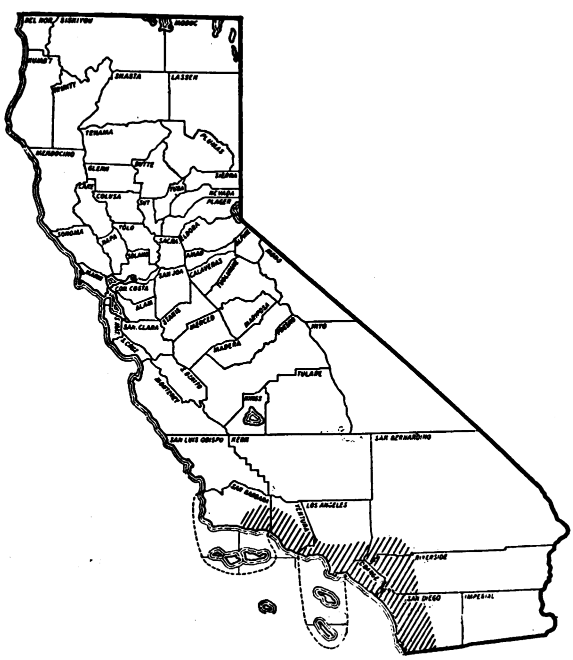


FIG. 1. Hatched portion indicates California citrus areas in which mesophyll collapse in orange leaves is prevalent.

though mesophyll collapse is seen in very young trees under conditions where no great stress on the transpiration system has occurred. Trees growing under optimum soil- and air-moisture conditions and subjected to occasional severe water deficits, generally show the greatest incidence of mesophyll collapse, while trees continually subjected to water deficit are least affected. In groves observed by us, leaves showing the disorder were found only on the north side of the affected tree, or they were generally distributed around the tree.

The prevailing opinion is that the necrosis following infestation with the citrus red mite, or red spider, *Paratetranychus citri* (McG.), is a forerunner or an accompaniment of mesophyll collapse. In fact, the distribution of citrus red mite in California, as shown by QUAYLE (**21**), is approximately the same as that of mesophyll collapse. Many trees not infested with citrus red mite are bady affected with mesophyll collapse, however.

The purpose of the present paper is to report studies made during 1941 and 1942 on the structure and composition of leaves of citrus trees affected with mesophyll collapse in southern California.

Materials and methods

For Ca, Mg, Na, K, Cl, and certain ash analyses, leaves were collected near Claremont, California, in early June and in late July, from Washington Navel orange trees growing on light sandy soil (Ramona loam), the pH of which, at field capacity, was 4.80 to 6.01 at the 1-foot level. A second sample of leaves for S and certain ash analyses was collected in August from the Washington Navel orange trees at Claremont and from Valencia orange trees at San Juan Capistrano. The Valencia orange trees were growing on heavy-textured Montmorillonitic yellow-clay soil (Diablo adobe clay), which had a pH of 6.15 to 6.39 at field capacity. In October, a third sample of leaves was collected from the San Juan Capistrano grove for S, P, and ash analyses.

Samples of injured and normal leaf tissue, of equal area, were excised from the leaves with a cork borer. For every sample of injured tissue, a sample of normal tissue was excised from a similar position on the blade, on the opposite side of the midrib.

Samples of Washington Navel orange leaf tissue, for sap analyses, were autoclaved 15 minutes at 15 lb. per square inch and pressed at 16,000 lb. per square inch. The sap and press cake analyses for Ca, Mg, Na, K, and Cl were made on 100 grams of sap and on 100 grams of press cake dried at 70° C., respectively. Methods of the United States Department of Agriculture Bureau of Plant Industry, Division of Irrigation Agriculture, Rubidoux Laboratory, located at Riverside, California, were used.

Analyses for total S as SO_4 were made according to the methods of the Association of Official Agricultural Chemists. Analyses for P as PO_4 were made according to methods of the Rubidoux Laboratory, using $(NH_4)_3PO_4 \cdot 12\ MoO_3$. All leaves for these analyses were samples as previously described. The samples were heated for 2 hours at 110° C. to inactivate the enzymes and were then dehydrated at 65° C., *in vacuo,* until the material attained constant weight.

Samples of affected and healthy areas of leaves collected in August from the same groves as the material for chemical analyses, were killed in formalin acetic acid alcohol and prepared for microscopical examination by the paraffin method. Sections 12 μ in thickness were stained with Delafield's hematoxylin and safranin, and permanent mounts were made.

Results

Results of a brief study of the distribution of collapsed areas in affected leaves of Washington Navel and Valencia orange are shown in table I. For this study, each leaf was divided into two portions, the outer or marginal portion and the inner or middle portion; the collapsed areas at proximal, central, and distal locations in these portions were counted. It was clear that the incidence of the disorder occurred principally in the central portion of the leaves.

Of the Washington Navel orange leaves which exhibited mesophyll collapse (table I), 17.2 per cent. were small, 54.4 per cent. were medium, and 28.4 per cent. were large. Of the Valencia orange leaves which exhibited mesophyll collapse, 31.7 per cent. were small, 40.0 per cent. were medium, and 28.3 per cent. were large. This is what would be expected from the normal size distribution of orange leaves. In the Washington Navel orange leaves, 68.7 per cent. of the translucent areas contained necrotic areas (brown spots); in the Valencia orange leaves, 40.3 per cent.

TABLE I

DISTRIBUTION OF AREAS AFFECTED WITH MESOPHYLL COLLAPSE IN ORANGE LEAVES, EXPRESSED IN PERCENTAGE OF TOTAL NUMBER OF COLLAPSED AREAS

LOCATION	WASHINGTON NAVEL		VALENCIA	
	MARGINAL LEAF PORTION	MIDDLE LEAF PORTION	MARGINAL LEAF PORTION	MIDDLE LEAF PORTION
	%	%	%	%
Proximal	4.0	8.0	9.3	16.3
Central	18.7	42.0	13.9	33.4
Distal	9.3	18.0	10.8	16.3

From a comparative histological examination of normal green leaf tissue and of affected leaf tissue, it was evident that the injured tissue contained collapsed spongy mesophyll cells (plate I, figs. 3–6). Until the affected areas dried, the palisade tissue was apparently normal (plate I, figs, 3–4). Intercellular space was reduced in the abnormal tissue, owing to the collapse of much of the spongy mesophyll and the enlargement of certain spongy mesophyll cells which had not collapsed. The diameters of the enlarged cells averaged 35 µ, whereas the diameters of normal cells averaged 18 µ. The walls of the enlarged cells had a maximum thickness of 2 µ; walls of normal sponge cells had a maximum thickness of 0.8 µ. Collapsed and injured tissue stained deeply with safranin, as shown by the dark regions in figure 4. The disorder was not confined to one vein islet, but included several to many.

Only rarely was the palisade tissue abnormal above the abnormal spongy mesophyll cells. As shown in plate I, figure 6, the palisade cells are shortened and not elongated. This suggests that, in addition to hypertrophy and collapse in the sponge tissue, on rare occasion hyperplasia may be observed in the palisade tissue, a condition reminiscent of boron deficiency. The palisade mesophyll directly above the affected spongy mesophyll is generally normal, however. This is confirmed by examination of tangential sections through palisade tissue above normal spongy mesophyll and above abnormal spongy mesophyll.

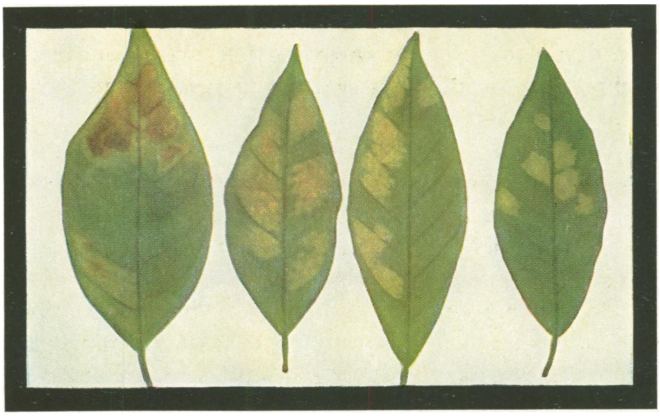
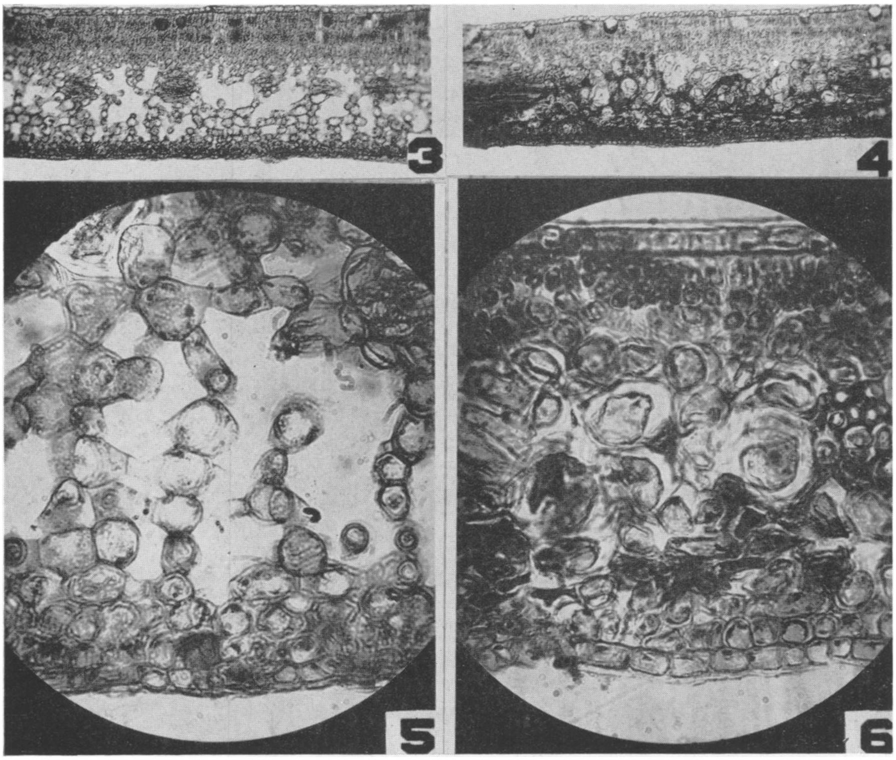


FIG. 2. Mesophyll collapse of sweet orange leaves photographed by transmitted light. (From color plate 27, Klotz and Fawcett, [19]).



Photomicrographs of transverse sections of Washington Navel orange leaves.

FIG. 3. Section through normal portion of leaf showing greater leaf thickness and large intercellular space. x 64.

FIG. 4. Section through abnormal portion of leaf showing reduced leaf thickness, collapsed mesophyll and abnormally enlarged mesophyll cells. x 64.

FIG. 5. Same section as Fig. 3. x 287.

FIG. 6. Same section as Fig. 4. x 287.

TABLE II

SULPHATE AND CARBONATE ASH IN COLLAPSED AND NORMAL LEAF TISSUE OF WASHINGTON NAVEL AND VALENCIA ORANGE, EXPRESSED IN GRAMS PER 100 GRAMS OF DRY SUBSTANCE

SAMPLE	LEAF TISSUE	SO_4 ASH	CO_3 ASH
WASHINGTON NAVEL			
		gm.	*gm.*
1	Collapsed	14.8	11.1
1c	Normal	16.8	11.4
2	Collapsed	17.8	11.2
2c	Normal	18.3	11.5
3	Collapsed	17.7	11.6
3c	Normal	18.9	11.2
4	Collapsed	18.4	11.3
4c	Normal	19.6	11.2
VALENCIA			
7	Collapsed	15.8	8.9
7c	Normal	17.8	13.5

The normal spongy mesophyll, as seen in tangential section, contains intercellular space. The abnormal spongy mesophyll, prior to collapse, shows hypertrophy of certain sponge cells so abutting that no intercellular space is visible.

While normal leaves vary greatly in thickness, sections of the same leaf at loci where normal tissue is found, and where mesophyll collapse is apparent, differ greatly. The averages of numerous measurements indicated that, in normal tissue, the leaf thickness was 283 μ, palisade thickness was 91 μ, and sponge thickness was 183 μ; while in abnormal tissue, the leaf thickness was 255 μ, palisade thickness was 87 μ, and sponge thickness was 153 μ. It was of especial interest to note that the cuticle was intact in all sections. Chloroplasts seemed to be fewer in number in the enlarged spongy mesophyll cells than in the normal.

TABLE III

DISTRIBUTION OF ALKALINE-EARTH AND ALKALI BASES AND OF CHLORIDE BETWEEN EXPRESSED SAP AND PRESS CAKE OF COLLAPSED AND OF NORMAL LEAF TISSUE OF WASHINGTON NAVEL ORANGE, EXPRESSED AS MILLIEQUIVALENTS PER 100 GRAMS OF SAP OR DRY SUBSTANCE, RESPECTIVELY

SAMPLE	LEAF TISSUE	EXPRESSED SAP					PRESS CAKE				
		Ca	Mg	K	Na	Cl	Ca	Mg	K	Na	Cl
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*
3	Collapsed	44	4	12	3	6	234	29	13	14	1
3c	Normal	68	1	10	1	3	256	11	8	5	1
4	Collapsed	44	3	7	3	3	236	22	8	6	1
4c	Normal	91	2	5	1	1	278	14	7	3	2

Chemical analyses indicate a number of differences between collapsed and normal tissue. As shown in table II, the sulphate ash in the dry matter of collapsed tissue was in every case lower than that in the dry matter of normal tissue. On the other hand, the carbonate ash was about the same in both normal and collapsed tissue.

The Ca content of the sap of collapsed tissue was lower than that of the sap of normal tissue (table III) but the Mg, K, Na, and Cl contents were higher in the sap of collapsed tissue than in that of normal tissue (table III).

The Ca content of the press cake of collapsed tissue, also, was lower than that of normal tissue (table III); and the Mg, K, and Na contents were higher in the press cake of collapsed tissue than in that of normal tissue (table III). There was no significant difference in the Cl content of the press cake from normal and collapsed tissue (table III).

TABLE IV

ALKALINE-EARTH AND ALKALI BASES IN COLLAPSED AND IN NORMAL LEAF TISSUE OF WASHINGTON NAVEL ORANGE, EXPRESSED IN MILLIEQUIVALENTS PER 100 GRAMS OF DRY SUBSTANCE

SAMPLE	LEAF TISSUE	Ca	Mg	K	Na	TOTAL Ca, Mg, K, AND Na
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*
1	Collapsed	177	19	63	5	264
1c	Normal	229	11	32	1	273
2	Collapsed	230	22	40	3	295
2c	Normal	258	13	23	3	297
3	Collapsed	245	30	15	15	305
3c	Normal	297	12	14	5	328
4	Collapsed	277	25	14	9	325
4c	Normal	382	15	13	4	414

Analyses of unexpressed normal and collapsed leaf tissues for alkaline-earth and alkali bases are shown in table IV. The analyses of samples 1, 1c, 2, and 2c are averages of three separate examinations; namely, analysis of plant ash, the sum of 30 per cent. C_2H_5OH-soluble and -insoluble fractions, and the sum of fractions soluble and insoluble in 0.05 N $BaCl_2$ in 30 per cent. C_2H_5OH. Analyses of samples 3, 3c, 4, and 4c are summations of analyses of expressed sap and of press cake. The results shown in table IV are in agreement with the direction of magnitudes of the cation contents in the sap and press cake analyses shown in table III; that is, the Ca is lower in the collapsed tissue than in the normal tissue, and the other cations are higher. The Na content of sample 2 presents the only exception: it was the same as that of the normal tissue of sample 2c.

Analyses made of the unexpressed normal and collapsed leaf tissues for total S as SO_4 and P as PO_4 are shown in table V. In samples 5 and 5c from Washington Navel orange leaves, and 6 and 6c from Valencia orange leaves, the normal tissue contained more S than the collapsed tissue. In the Valencia leaves of samples 7, 7c, 8, and 8c, however, the S content of the collapsed tissue was higher than that of the normal tissue. In all cases, the P values were higher in the collapsed tissue than in the normal tissue. Semiquantitative microanalyses in samples 5, 5c, 6, and 6c indicated larger concentrations of P in collapsed tissue. The percentage of moisture in collapsed and normal samples of leaf tissue are also shown in table V. The normal leaf tissue of Washington Navel orange and of Valencia orange contained a higher percentage of moisture than the collapsed tissue. The pH of normal leaf sap was 5.42, which was slightly, but not significantly, higher than the pH of sap from collapsed leaf tissue, which was 5.37.

TABLE V

PERCENTAGE MOISTURE, TOTAL SULPHUR, AND TOTAL PHOSPHORUS IN COLLAPSED AND NORMAL LEAF TISSUE OF WASHINGTON NAVEL AND VALENCIA ORANGE, EXPRESSED IN MILLIGRAM ATOMS PER 100 GRAMS OF DRY SUBSTANCE

SAMPLE	LEAF TISSUE	MOISTURE	S	P
		WASHINGTON NAVEL		
		mg. atom.	*mg. atom.*	*mg. atom.*
5	Collapsed	47.10	7.3*	3.8
5c	Normal	48.16	8.3*	3.0
		VALENCIA		
6	Collapsed	41.87	9.2*	3.5
6c	Normal	42.83	12.1*	3.1
7	Collapsed	....	23.5	2.9
7c	Normal	....	20.5	2.3
8	Collapsed	....	28.2	2.8
8c	Normal	....	23.6	1.7

* The authors wish to thank PROFESSOR A. J. HAAGEN-SMIT, California Institute of Technology, for the microanalyses reported here.

Comparison of our leaf analyses with those of other workers dealing with various nutrient deficiencies and excesses, indicated that the principal differences between their results and ours lay in the high Mg values obtained by us. Consideration of the soil types where mesophyll collapse occurred in California, suggested that Mg^{++} might be associated with the disorder.

Several experiments were run with potted sour-orange seedlings, which were watered with 50 e.p.m. of $MgSO_4$ solution for two weeks and then leached with distilled water. After two days of the latter treatment, leaf symptoms of mesophyll collapse developed. The leaves were sectioned and examined. Similarity to mesophyll collapse was evident.

Discussion

Ignited residue weighed as carbonate ash is obtained at the relatively low temperatures of dull-red heat. It contains, chiefly, combinations of carbonates, oxides, and phosphates of the alkaline-earth and alkali bases; small amounts of Si, SO_4, and Cl; and other quantitatively minor constituents.

Organic compounds of the bases, such as $CaC_2O_4 \cdot H_2O$, for example, and nitrates are converted into carbonates or oxides in the course of the combustion.

Sulphate ash, on the other hand, is obtained at much higher temperatures. The plant material, prior to the combustion, is treated with H_2SO_4. Under such conditions the ignited residue consists largely of sulphates and phosphates. As the equivalent combining weight of SO_4 is higher than that of CO_3 or O, correspondingly higher figures for the SO_4 ash are obtained, all other things being equal.

In view of these considerations, it is possible to interpret the results shown in table II in the following manner. Organic compounds of the bases are less prominent in the collapsed tissue than in the normal tissue, as evidenced by the consistently smaller differences between the SO_4 and the CO_3 ash of the collapsed tissue. This conclusion is also supported by the fact that SO_4 ash is higher in the normal tissue than in the collapsed tissue, while carbonate ash, in all samples except those of Valencia orange, is nearly identical in the two tissues. Furthermore, were the CO_3 ash residues largely identical in kind, the trends in the SO_4 ash would not have become manifest. One may infer, therefore, that, while some quantitatively important "acidic" constituents of the CO_3 ash of collapsed tissue are similar in their equivalent combining weight to those of the CO_3 ash of normal tissue, they are not converted to SO_4 in the course of the SO_4 ash determination. Tertiary PO_4 would be the most likely type of substance responsible for this difference. If it is assumed that the observed difference is due to tertiary PO_4, the collapsed tissue would be expected to contain more PO_4 than the normal tissue, but less of the organic compounds of the alkaline-earth and alkali bases. Actually, analyses of the tissue for PO_4 showed that this was the case.

Relative amounts of Ca in the sap phase of collapsed tissue are much lower than in the corresponding samples from normal tissue. The ratio of Ca to the alkali bases, for example, is 3 and 4 in the sap of collapsed tissue as against 6 and 15 in that of normal tissue, for samples 3 and 4 and 3c and 4c, respectively. In the press cake analyses, similar trends are also manifest; but, recognizing that variable amounts of sap are retained in the press cake against a pressure of 16,000 lb. per square inch, we have not relied on the press cake analyses alone.

Hydration of colloids contained in the leaf tissue is most probably influenced by the kind of cations combined or associated with the colloids. It is reasonable to infer that, all other things being equal, any increase in the proportion of monovalent cations, that is, K^+ and Na^+, in the liquid phase in equilibrium with the colloidal surfaces, would produce corresponding changes in the cations adsorbed by the colloids; whereupon the extent of hydration of the latter would in all probability be increased. In fact, STUEWER (**23**) showed that, in aqueous solution, pectic acid is 21 per cent. hydrated; pectin, 25; calcium pectate, 35; and sodium pectate, 38. Such an increase in hydration would also be brought about by the removal of Ca^{++} by replacement with monovalent cation.

Precipitation or translocation of Ca^{++} would be expected after replacement, and our analyses suggest the latter possibility rather than the former. Any increase in amounts of cations other than Ca^{++}, would tend to increase the mobility of Ca^{++} in the plant tissue, whether this Ca is in the adsorbed or in the precipitated form.

STUEWER (**23**), as a result of his experiments, states that the sodium salts of pectin are highly dissociated, that the salts of divalent metals are considerably less dissociated, and that the acid is still less dissociated. These results are in agreement with the reported facts that sodium salts hinder jelling, while calcium chloride promotes it. In our own studies, despite variations in the absolute amounts of the bases and in every comparison of collapsed tissue with corresponding normal tissue, Mg, K, and Na were always higher in the collapsed tissue than in the normal tissue, while Ca was always lower. This suggests that the weakening of the gel structure in the spongy mesophyll cells may be caused by the high concentrations of Mg^{++}, K^{+}, and Na^{+}, and the low concentrations of Ca^{++}, so that in irregularly occurring periods of unusual transpirational stress, collapse of the cells results.

The work of KERTESZ *et al.* (**18**) seems to support this interpretation of results. They found that Ca salts added to canned tomatoes result in the better retention of the original firmness of the fruit. It was postulated by KERTESZ (**17**) that the addition of Na or K salt accomplished just the opposite of the Ca effect, inasmuch as Ca is removed from the tissue by an exchange of ions. This reaction was confirmed by GREENLEAF, according to KERTESZ *et al.* (**18**). GREENLEAF (**13**) has further shown that the Ca salt improves the texture of the canned product by forming calcium pectate.

The findings of STEWARD, STOUT, and PRESTON (**22**), in their respiration studies, indicate that K^{+} increases the mobility of pectin. They found that KBr at high oxygen tension, through the action of K^{+} on Ca^{++}, caused the transfer of a uronic acid from potato disks to blotting papers; this, when calculated as pectin, accounted for the concomitant loss of carbon on their balance sheets. It also seems possible that such colloids as proteins might act in a similar manner under the influence of K^{+} or Na^{+}.

The fact that normal leaf tissue has a higher percentage of moisture than tissue containing collapsed spongy mesophyll cells, may be interpreted as support of the explanation of the mechanism of collapse, since weakening of the gel structure to the extent where collapse would occur would be followed by a loss in water and necrosis. This process is commonly observed as a result of freeze injury in citrus fruits and affects entire vesicles.

While these facts suggest the mechanism of the collapse of the spongy mesophyll, they do not contain any suggestion as to the mechanism of the enlargement of certain of the spongy mesophyll cells found among the collapsed cells.

The theory that the citrus red mite, or red spider, may be responsible for mesophyll collapse seems to offer an explanation of the enlarged cells, since the effect of certain indole derivatives and excreta from insects is known to cause intumescences in leaves (**20**). Mesophyll collapse may be abundant, however, where red spider infestations are light; and in greenhouse studies with red spider, we failed to produce symptoms of mesophyll collapse. Macroscopic examination of the surface of certain leaves from the field, exhibiting mesophyll collapse, failed to show symptoms of red spider injury, such as silvering of the upper surface of the leaf. Microscopic examination in cross section failed to show spots in the palisade parenchyma lacking chlorophyll, which we have found in silvered leaves having heavy infestations of red spider. We have therefore questioned the red spider explanation of mesophyll collapse, despite the similar distribution of the two.

HAAS and THOMAS (**15**) have shown that in leaves from Lisbon lemon trees, severe injury is correlated with a high S content ranging from 0.68 to 1.10 per cent. of the dry matter. In the field, SO_4 injury occurred where $NaNO_3$ was absent in fertilizers containing blood and bone meal and K_2SO_4; or blood, bone meal, and K_2SO_4; or impure $CaH_4(PO_4)_2$ (superphosphate), alone or in combination with organic fertilizers, but without $NaNO_3$. The SO_4 content of mesophyll-collapsed tissue, however, is about normal. Values agree well with those reported by KELLEY and CUMMINS (**16**) for normal leaves and with those reported by HAAS and THOMAS (**15**) for uninjured leaves. But our values are much lower than those reported by HAAS and THOMAS for leaves showing severe SO_4 injury.

Analyses of CHAPMAN and BROWN (**2**) on leaves of P-deficient navel-orange trees, show a disturbed ionic balance in the leaf tissue similar to that found by the authors in mesophyll collapse, except that the P content was low in their material and high in ours. The values of CHAPMAN and BROWN (**3**) for S-deficient plants, as compared with healthy plants, agree with our values for collapsed tissue of mature leaves, as compared with normal leaf tissue, except in S content, where our results were variable. The values of CHAPMAN and LIEBIG (**4**) for high-SO_4 plants at various nitrogen levels are similar to our results for Ca, K, and P; but Mg is low and S is high for high-SO_4, while in mesophyll-collapsed tissue, Mg is high and S is variable. Only a few points of agreement were noted between their chloride series and our results.

EATON (**5**) grew eight crops in nutrient media containing 50 and 150 milliequivalents of Cl and 50, 150, and 250 milliequivalents of SO_4 per liter. His sap analyses of these crops for Ca, Mg, K, and Na, as well as the sum of the cations in several instances, show the same trend for high SO_4 plants, as compared with control, as our results show for collapsed tissue as compared with normal tissue.

From the preceding comparisons, it may be surmised that mesophyll collapse is related chemically to an unbalance in the ionic constituents of the plant. It is possible that this unbalance may be due to excesses or deficiencies in soil constituents.

Morphologically, the appearance of mesophyll collapse may resemble the external leaf symptoms sometimes obtained in water culture in B deficiency or in cultures at high nitrate levels, using $Ca(NO_3)_2$. Certain leaves showing symptoms of mesophyll collapse were obtained from such cultures[3] for histological examination. None of these leaves contained collapsed spongy mesophyll cells, but they showed distinct histological characteristics peculiar to the two types of cultures from which they were obtained. CAMP[4] states that he has produced symptoms of mesophyll collapse by treating dry citrus trees with excesses of KNO_3, or with $Ca(NO_3)_2$. BAIN and CHAPMAN (**1**) produced similar symptoms in grapefruit cultures, using $Ca(NO_3)_2$ in combination with waterlogged soil.

Further work will be necessary to determine whether one or several types of ionic unbalance may bring about typical external and internal symptoms of mesophyll collapse.

Summary

Mesophyll collapse apparently occurs in leaves of orange trees in the coastal regions of all southern California orange districts. It is confined largely to leaves of orange and has seldom been seen on those of lemon. Leaves exhibiting mesophyll collapse have chlorotic, translucent, water-soaked-appearing areas, principally in the central portion of the leaf blade, although collapse symptoms may occur anywhere in the lamina; brown necrotic spots may develop in the translucent areas. Histological examination of affected leaf tissue shows enlarged sponge cells interspersed with collapsed sponge cells, reduced intercellular spaces, and chlorophyll-depleted spongy mesophyll cells.

Ca was invariably found to be lower in collapsed tissue than in normal tissue, while K, Mg, Na, Cl, and P were somewhat higher than in normal tissue. The ratio of Ca to K was accordingly lower in the collapsed tissue than in the normal tissue. Distribution of the alkaline-earth and alkali bases between the liquid (sap) and the solid phases of autoclaved leaf tissue likewise showed similar differences in absolute and in relative amounts of Ca and K. No consistent difference in the S content of collapsed and normal tissue was found. Consistently lower percentages of SO_4 ash were found in the collapsed tissue than in the normal tissue, while the CO_3 ash was the same in both normal and collapsed tissue.

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